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Bioactive Bromopolyacetylenes From The Marine Sponge *Xestospongia Testudinaria*

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Abstract: Xestospongic acid **1a** and its ethyl ester **2**, bioactive bromopolyacetylenes, have been isolated from the marine sponge *Xestospongia testudinaria*. Their structures have been assigned by spectral methods. Both are antimicrobial and compound **2** is a Na^+/K^+ ATPase inhibitor.

Marine sponges of the genus *Xestospongia* have been a fertile source of various original secondary metabolites, oxaquinolezidines[1], polycyclic quinones[2,3], β-carboline alkaloids[4], terpenes[5], lactones[6], aminoalcools[7], as well as polyacetylenic derivatives[8-10], with potential biological activities.

The dichloromethane/methanol extract of the fresh sponge *Xestospongia testudinaria*, collected by scuba at Mayotte, showed antimicrobial activity against *S. aureus* . Preliminary separation on silica gel yielded two active fractions eluted with chloroform and chloroform/methanol 9/1 respectively. Successive separations on silica gel and LH20 of the most polar antimicrobial fraction led to isolation of the active and unstable acid **1a** (I.R. νC=O 1695 cm^{-1})(0.05 % wet weight), which was difficult to obtain in pure form. Methylation of **1a** by diazomethane, followed by repeated silica gel chromatography (hexane/EtOAc 9/1) furnished the major product **1b**, as a yellow oil, M^+: 362/364 (E.I.).

The molecular formula $C_{19}H_{23}O_2Br$ of **1b** was established by HRMS (M^+: 362.0872, calcd. for $C_{19}H_{23}O_2{}^{79}Br$: 362.0881). I.R. absorptions at 2216 and 1741 cm^{-1} suggested acetylenic bond(s) and an ester group.

The ^1H NMR data exhibited signals for two *trans* ethylenic protons (δ 6.55 ppm; J = 14.0 Hz, 0.6 Hz and 6.18 ppm, J = 14.0, 2.3 Hz), a methoxyl group, and nine methylene protons. The small 0.6 and 2.3 Hz couplings indicated an interaction across an acetylenic bond, which was confirmed by ^1H-^1H COSY data, suggesting the fragment: Br-CH = CH-C≡C-CH_2-.

The ^{13}C NMR spectra of **1b** (BB and *J* Mod) confirmed these indications and showed one carbonyl, two ethylenic carbons, a methoxyl, six signals (δ 92.99-65.21 ppm), which could be assigned to acetylenic carbons, in accordance with the IR spectrum and the formula, six methylene groups (δ 32.70 to 23.50 ppm) and three shielded signals attributed to methylenes adjacent to acetylenic carbons[10].

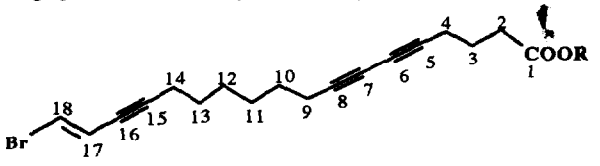
Both ^1H-^{13}C CORR LR (optimized with J=7Hz) and ^1H-^{13}C CORR (4K x 1K) experiments with increasing resolution permitted unequivocal assignments of all carbons except C-11 and C-12 since H-11 and H-12 are superimposed (Table 1). On the basis of these data, structure **1** was deduced for xestospongic acid. Acid **3** was previously reported as a major constituant from *X.testudinaria*[10] collected in Australia (0.1% wet sponge); this discrepancy might be due to interspecific variability in the species.

Repeated chromatography of the less polar fraction on silica gel (hexane/EtOAc 9/1) afforded **2** as a pale yellow oil. Compound **2** was analyzed for the molecular formula $C_{20}H_{25}O_2Br$, in agreement with its molecular ion peak at m/z 376/378. ^1H NMR of **2** showed peaks identical with those reported for **1b**, except for the absence of the methoxyl signal and the presence of a quartet at δ 3.66 ppm and a triplet at δ 1.23 ppm, indicating an ethoxy group. Hence, structure **2** was assigned as the ethyl ester of xestospongic acid.

Compounds **1b** and **2** showed weak antimicrobial activity against *S. aureus*: Ø inhibition 12 mm and 15 mm at 500 μg/disc. Xestospongic acid **1a** is the most active: Ø inhibition 12 mm at 100 μg/disk.

On screening for enzymatic activity, compound **2** was found to inhibit the Na^+/K^+ ATPase, according to the method described by Hosie[11]: $ID_{50}=10^{-4}$-10^{-3} M.

Several polyacetylenic acids or alcohols isolated from sponges or other marine organisms have previously shown a variety of biological activities ranging from antibacterial, cytotoxic to enzyme inhibitors[12,13].



1a R = H
1b R = CH_3
2 R = CH_2-CH_3

3

Table 1: ^{13}C (75.47 MHz) and ^1H (300.13 MHz) NMR Spectral Data for **1b** ($CDCl_3$, δ ppm)

Position	δ ^{13}C (mult.)	^1H-^{13}C CORR*	δ ^1H (mult., *J* Hz)	^1H-^1H COSY
1	173.39 (s)			
2	32.70 (t)	- - - - - - - -	2.42 (t, 7.4)	
3	23.50 (t)	- - - - - - - -	1.80 (tt, 7.4, 6.9)	
4	18.67 (t)	- - - - - - - -	2.30 (*tt*, 6.8, 0.9)	
5	76.01 (s)			
6	66.15 (s)			
7	65.21 (s)			
8	77.73 (s)			
9	19.11 (t)	- - - - - - - -	2.26 (tt, 6.8, 0.9)	
10	28.08 (t)	- - - - - - - -	1.47 (m)	
11	28.24 (t)**	- - - - - - - -	1.36 (m)	
12	28.27 (t)**	- - - - - - - -	1.36 (m)	
13	28.16 (t)	- - - - - - - -	1.47 (m)	
14	19.36 (t)	- - - - - - - -	2.23 (ddt, 6.8, 2.3, 0.6)	
15	92.99 (s)			
16	77.33 (s)			
17	117.99 (d)	- - - - - - - -	6.18 (dt, 14.0, 2.3)	
18	117.02 (d)	- - - - - - - -	6.55 (dt, 14.0, 0.6)	
19	51.62 (q)	- - - - - - - -	3.65 (s)	

* - - : XH CORR, — : XH CORR LR, s: strong, w: weak; ** may be reversed.

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